Key technologies for LSPR-sensing microfluidic biochip

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ABSTRACT

New structures of micro-pump, biosensor in LOC (Lab-on-a-Chip) are studied. A round-path-chamber pumping principle is proposed to depress output fluctuation and increase output pressure of micro valve-less mechanical pump. Its prototype produces higher output pressure than the one with traditional column chamber. Two types of metal nanostructure array for LSPR (Localized Surface Plasmonic Resonance) biosensor were fabricated and tested, showing high sensitivity. Micro-channel is also discussed for realizing laminar flow above the metal nano-particles, to enable analytic samples to contact the metal nano-particles fully and stably.

Key words: LOC (Lab-on-a-Chip), LSPR (Localized Surface Plasmonic Resonance), micropump, biosensor, micro-channel

1. INTRODUCTION

LOC (Lab-on-a-Chip) technology has been developed quickly recent years. It is expected to play important role to deal with the threats to human being from pollution, diseases and terrorism[1-3]. Accurate microfluidic actuation and bio-detection of high sensitivity are two key technologies. Detection should show high sensitivity, efficiency and reliability. For testing by binding the molecules on biosensor, the actuation should not show any discriminating and destroying effect on components in the fluid.

However, Traditional bio-detecting principles usually employ marking materials such as enzyme, radioactive isotope and fluorescent dyes to indicate bio-molecules, suffering from complicated operation and inadequate stability. Two most popular pumping principles at present are not good for these requests, for electroosmotic pump separates the components[4-6] and the valve in a mechanical pump may destroy cells and big molecules[7-9].

In this paper, a new mechanism of micro mechanical valveless pump and two nano-structures of biosensor based on LSPR (Localized Surface Plasmonic Resonance) principle are introduced. The new pumping mechanism gets rid of the disadvantages above, and the biosensor would show high sensitivity, mark-free, real-time detecting ability and other advantages[10-16]. Two technologies would lead to the valuable combination of LSPR and LOC.

2. PRINCIPLE & STRUCTURE

2.1 New mechanism for micro mechanical valveless pump

In a microfluidic biochip, the bio-fluid is driven with a micropump to flow along micro channels, to pass over the sensor and to discharge out of chip finally. Part of the molecules in the fluid will react and be bound onto the surface of the biosensor. Eectroosmotic pump and mechanical pump with valves are most popular now since they produce very high pressure. An electroosmotic pump could separate the components in the pumping path, which is favorable for component abstraction and for detection by analyzing component spectrum. But the separation is undesired for bio-detection by binding molecules on biosensor. In a traditional mechanical pump, the valves may destroy cells and big molecules, making it unsuitable for most bio-detecting applications. So, we choose micro mechanical valveless pump to drive fluid because it has not any discriminating and destroying affection on the bio-component.

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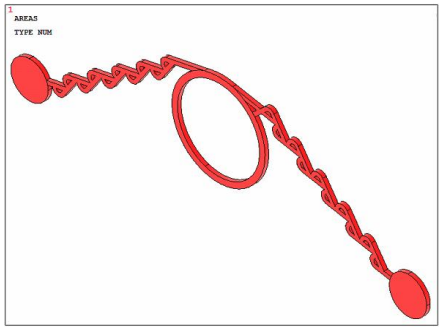
The output pressure of a micro mechanical valveless pump is not high and carries fluctuation [17-21]. So, we proposed a new pump structure characterized by a round-path-chamber to improve the performance. Its FEA (Finite Element Analyzing) model is depicted in Fig.1 and the hydrokinetic simulation result is showed in Fig.2. The pump is actuated by the deformation of a piezoelectric element attached on the membrane of the chamber. In the simulation, it is assumed that the membrane vibrates at 500Hz frequency and 32μm amplitude. Two "valvular conduits" invented by Nicola Tesla[22] are used for producing different impedances in two flow directions.



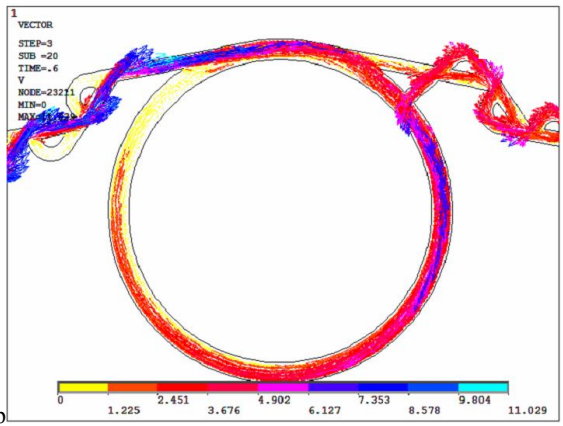
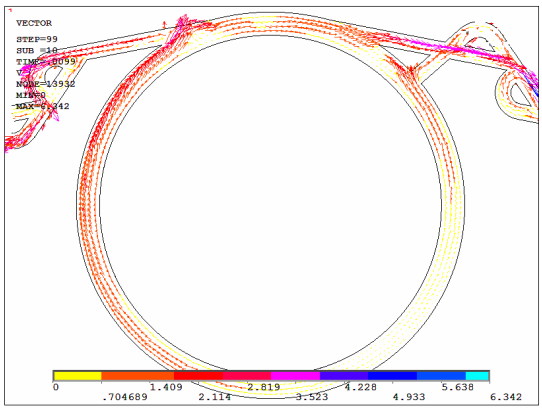


Fig.1 FEA model of the micropump

a. Clockwise momentum predominance in the expanding process of chamber (dense vector marks is used)



b. Keeping of the predominance in the contracting process of chamber

Fig 2. Simulation of pumping mechanism

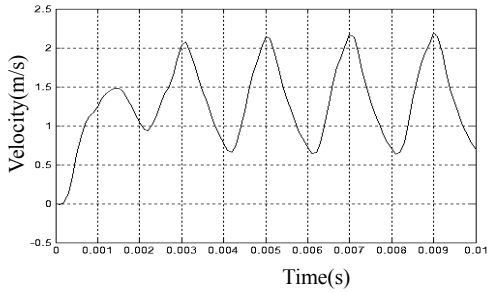


Fig.3 Velocity on a point near the chamber outlet

The principle of this structure relies on the establishing and strengthening of momentum predominance in one circular direction (Fig.2). In the expanding process of chamber, clockwise momentum predominance is established (Fig.2a). And in the contracting process of chamber, the flow in most part of the circular path will be kept in clockwise direction (Fig.2b). Vector marks in Fig.2a is much denser than Fig.2b in order to emphasize the clockwise momentum predominance.

Such momentum predominance reduces the proportion of back flow in the chamber that causes output fluctuation. It can also increase the difference between resistances in two directions, prevent the chamber from containing any bubbles that limits the pressure output by leading to fogging phenomenon. So, the pressure output will increase and the fluctuation will be depressed. The velocity changing on a point near the outlet in the chamber is showed in Fig.3. Backward flow that is common in a tradition chamber does not occur here, although fluctuation is still obvious. The simulation results also suggest that the clockwise momentum predominance will be strengthened after every cycle. So, both the pressure and the output stability will be improved continuously.

2.2 Design of micro-channels

The proper design of micro-channel is also important for the performance of the chip. Although the flowing velocity can be adjusted by controlling micropump, the sensing accuracy could still be affected by turbulence, sedimentation, fluctuation of pressure and other factors. Proper micro-channel structures that can form steady laminar flow make testing process exactly comparable, and the data reliable. However, the flow above nano-structure is difficult to deal with. Biosensor is usually designed to be located in a round column volume since the sectional shape of light beam is usually round. When the fluid flows through such a chamber, vortexes may occur under instable pressure and result in turbulence that is undesirable. Moreover, low flux on the points far from the inlet or outlet ports leads to difficult cleaning and low bonding efficiency in this area. So, the traditional shape of biosensor container should be changed.

The second benefit of optimizing micro-channel structure is the increase of bonding probability between object molecules and the metal surface, which in turn improves sensitivity. For this purpose, the shape and dimension of the volume above the nano-particle array should be controlled exactly in order to make the fluid pass along the laminar layer proximate to the metal surface, to enable analytic samples to contact the metal nano-particles fully. This is more convenient and efficient than optimizing the metal nano-structure array. The latter is the common way now, but limited by lack of low-cost fabricating process.

2.3 Bio-sensing principle of the nano-structure

The bio-molecules are captured when passing over the metal nano-structure. A layer of probing molecules has been previously bound on the metal surface. The molecules in the fluid will react with probing molecules and then be captured, changing the refractive index above the nano-structure. On the surface of metal nano-structure in an electromagnetic field, there is an electron density wave that acts as a polarized vibrating vector. When the vector of incident light along the particle surface matches the inherent vector, LSPR resonance occurs. The energy of the incident light will be absorbed into the metal, resulting in obvious drop of the reflecting or transmitting energy, corresponding to a peak in extinction spectrum or a valley in penetration spectrum.

The captured bio-molecules change the refractive index around the nano-particles, influencing the LSPR condition and changing the frequency of LSPR. It will be seen in the spectrum of transmission or distinction. Different captured

molecular corresponds different shift of the peak or valley in the spectrum curve. By designing particles of different shape, dimension and period, the LSPR results could be different.

Two kind of biosensor structure are studied in our group now. One is a metal nano-particle array on a quartz plane, and the other is a porous membrane of aluminum oxide. They work all on the LSPR principle. The nano-structures are often less than 100nm in dimension, distributed periodically. The array could take various patterns, determined by the fabricating techniques[10].

3. FABRICATION & TEST RESULTS

The pump prototype (Fig.4) consists mainly of a thin glass membrane, a piezoelectric element and a silicon substrate in which the micro-channels of 50μm wide and 100μm deep are made. The membrane and the substrate are bonded together under high pressure and temperature. The piezoelectric element is attached on the membrane with epoxy glue, powered by sinusoidal signal of ±200V. In the test, deionized water at 15℃~20℃ is used for water is main body of most bio-analyzed microfluid. The prototype of the pump shows maximal flow of 35×10^{-6}L/min and maximal output pressure of 1.52kPa. The curve in Fig.5 shows that , the pressure of this pump is higher than that of a pump with a traditional column chamber in same dimension. In the test, the water level in outlet tube seems extremely stable under microscope.

The first nanostructure, metal nano-particle array (Fig.6), is made with NSL (Nano-sphere Lithography) technology[10]. The reagent containing polystyrene nano-spheres is dropped onto a quartz plate. Along with the volatilization of reagent, polystyrene spheres arrange themselves into a regular compact two-dimensional array. Then, vaporized heavy metal (Au, Ag, Cr) deposits not only on the surface of spheres, but also onto the surface of quartz through the gaps between spheres. By choosing diameter of spheres and adjusting depositing time, the dimension and period of the metal nano-particles could be controlled. In reference[10], ultrasonic vibration is employed finally, so the nano-spheres broken-off and only an array of metal nano-particles remain on the surface of quartz. But in our experiment, the nano-spheres remain also on the quartz because no ultrasonic vibration was used.

The second nano-structure, porous Al_2O_3 membrane (Fig.7), is made by chemical nano-assembling technology. Then we modified the surface and deposited noble metal on both side of the membrane (Fig.8).

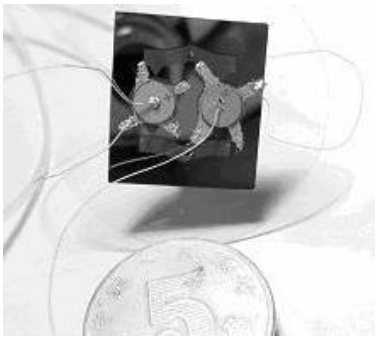


Fig.4 One of the micro-pump prototype

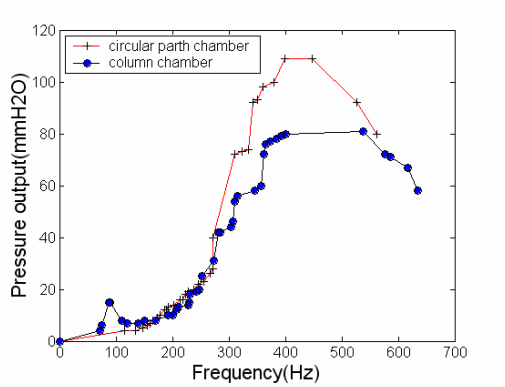


Fig.5 The output pressure-frequency curve of the micropump

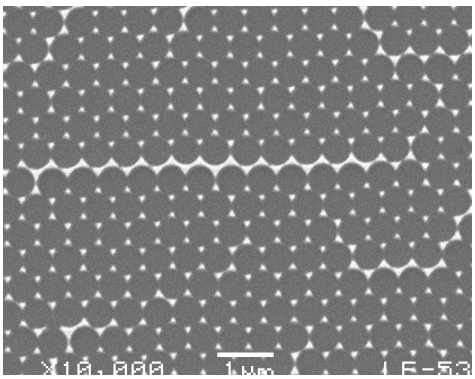


Fig.6 SEM picture of Ag@ polystyrene sphere(diameter 260nm) array

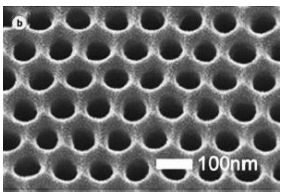


Fig.7 SEM picture of the porous Al_2O_3 membrane (thickness 10 μm)

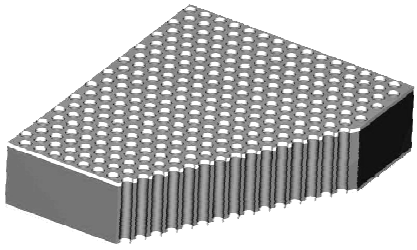


Fig.8 Membrane deposited by Ag (thickness 30nm on both sides)

Since the biosensor principle is based on the change of refraction index on the metal surface, we coat a layer of inorganic colloid of about 7nm on the array. The colloid consists of silicon dioxide and titanium dioxide. Their proportion can be adjusted to make different refraction index. Fig.9 and Fig.10 show the transmission spectrum curves. Narrow and sharp peaks indicates high sensitivity..

The wavelength data of right peak, given on the top right corner of Fig.9, correspond respectively to different covering layer of air, colloid layer of refraction index 1.5 and that of refraction index 1.8. The sensitivity of peak and valley could be estimated with parameter *K*. *K*= the maximal shift divided by half width of the peak or valley when refraction index is 1. By calculation, *K* is 0.236, 0.77 and 0.93 respectively for right peak, left peak and valley. The valley corresponds to the peak in extinction spectrum. In Fig.9, along with the increasing of refractive index, the sensitivity degrades and the wavelength of peak and valley get bigger.

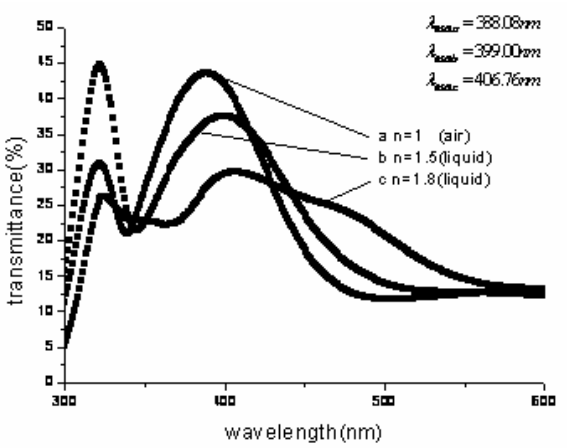


Fig.9 The transmission spectrum on different refractive index

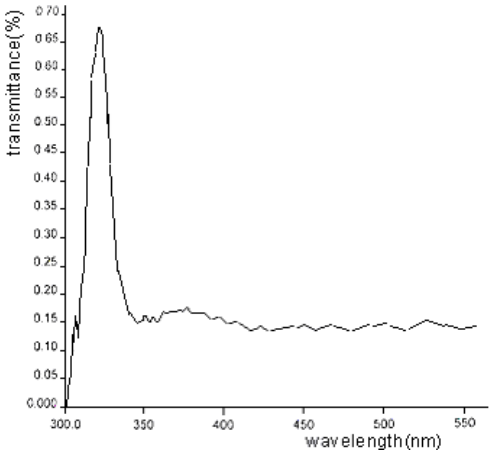


Fig.10 The transmission spectrum of a porous Al2O3 membrane

It can be found from Fig.10 that the transmittance of the light through the porous Al_2O_3 membrane is very low that may be the result of big thickness. It is not good for bio-detecting application and can also affect the sensitivity. However, the sharp peak of the curve indicates such a structure may show very high sensitivity. Our attention is paid now more on the enhancement of the transmittance than on the shift of peak frequency. The membrane of less thickness will be fabricated and tested.

4. CONCLUSIONS

LSPR bio-sensing nano-structures and suitable microfluidic pumping mechanism are studied. LSPR is very sensitive to the change of the refractive index on the surface of metal nano-particles. Since a LSPR biosensor works without bio-marking materials such as radioactive isotopes and fluorescent dyes, the objective molecules get the benefits of keeping bioactivity, the operation is simplified and the test data become more believable.

By combining LSPR and LOC technology, high sensitivity and high efficiency could be attained simultaneously, In a LOC biochip, real-time ability of LSPR, that is important for discovering interplaying detail among different molecules and cells, could be exploited. And LSPR biosensor could be cleaned automatically after every test, making the chip reusable and low-cost. Since the most popular micropump are not suitable for the chip with a LSPR biosensor. We proposed a new pumping mechanism of micro mechanical valveless pump. With a round-path-chamber, the pressure output increased and the output fluctuation is deepressed. By establishing and strengthening of momentum predominance in one circular direction, this structure makes the flow in chamber stronger and more stable just like an inertia flywheel.

In further research, we will bind bio-molecules on biosensors to get transmission spectrum, improve pressure output of the new pumping mechanism and find more diversiform nano-particles to improve sensitivity.

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